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## Scientific Note

# LOW SUSCEPTIBILITY OF OVERWINTERING MONARCH BUTTERFLIES TO *BACILLUS THURINGIENSIS* BERLINER

The large winter aggregations of the adult monarch butterfly, *Danaus plexippus* L. (Danaidae: Lepidoptera), are spectacular phenomena that occur among selected groves along the California coastline and in the high mountains of Mexico. The overwintering areas in Mexico are periodically infested by larvae of *Evita hyalinaria* (Grossbeck) (Geometridae: Lepidoptera), which defoliate the oyamel fir trees, *Abies religiosa* Lindley, used by the butterflies. Foliage protection can be attained with the use of *Bacillus thuringiensis* Berliner, but the application of this biotic agent may inadvertently affect the monarch butterflies overwintering in the sprayed region. Brower expressed concern over the widespread application of *B. thuringiensis* because of its potential negative effects on the monarch butterflies (Brower, L. 1986. Atala, 14: 17–19). He felt that large scale spraying of this biotic agent should be avoided near an overwintering site unless the procedure could be determined as safe or of minimal risk to the butterflies. Because the susceptibility of adult monarch butterflies to *B. thuringiensis* is unknown, we tested this bacterium against overwintering butterflies under laboratory conditions.

Butterflies were collected from a central coast wintering site located in Oceano, California (Leong, K. L. H. 1990. Ann. Entomol. Soc. Am., 83:906–910) and held in cages without water at room temperature and humidity (21° C ± 0.25 SE, 42% RH ± 1.1 SE) for two days before treatment. The butterflies (10 insects per cage and three cages per treatment) were initially subjected to twice the recommended concentration of commercial *B. thuringiensis* [*kurstaki*] (Javelin®, 1.9 liters/3758 liters of $H_2O$ [16,000 *Spodoptera* units/mg]) or to twice the recommended concentration of dead transgenic *Pseudomonas fluorescens* (Trevisan) Migula containing endotoxin crystals (MVP®, 1.9 liters/3758 liters of $H_2O$ [10,000 Diamondback units/mg]). Control groups were provided water only. Adult monarchs were exposed to *B. thuringiensis* by two ways: (1) spraying an aqueous suspension onto the leaves of *Eucalyptus* sp. (tree species commonly used by overwintering butterflies in California) within the cages until runoff (approximately 16 ml), and (2) placing an aqueous suspension in petri dishes (50 ml) for 24 h.

The adults imbibed the preparation almost immediately after exposure. Five days after exposure, the adults showed a low mortality to both Javelin® and MVP®. The butterflies exposed to Javelin® spray, however, had a significantly higher mortality ($P < 0.05$) than those sprayed with MVP® (20% ± 7 SE for Javelin® vs 3% ± 3 SE for MVP®). The same relationship, but not significantly different, was exhibited between the two groups of butterflies exposed to the inocula in petri dishes (7% ± 5 SE for Javelin® vs 3% ± 3 SE for MVP®). None of the control insects died during the study period. The results suggest that the butterflies were slightly more susceptible to *B. thuringiensis* preparation than to the transgenic *P. fluorescens*.

To confirm the results of the *B. thuringiensis* preparation, another test was conducted with Javelin® at the recommended (0.9 liters/3758 liters of $H_2O$) and at twice the recommended concentrations by spraying or by placing the aqueous suspension in petri dishes (10 insects per cage, three cages per treatment). The control adults were exposed to water only. The butterflies again exhibited low total mortality rates at both the recommended and twice the recommended concentration rates (7% vs 7% spray; 3% vs 7% petri dishes). The total mortality among the control insects was zero for the spray and 3% for petri dish treatment. *Bacillus thuringiensis* was isolated from the hemocoel of the dead butterflies treated with Javelin, but not from the controls.

The fecal droppings of butterflies treated with twice the recommended concentrations were collected daily and placed in 5 ml of sterile water and agitated. A loopful of the suspension was then streaked onto nutrient agar and incubated at 25° C. *Bacillus thuringiensis* isolates were determined by colony growth characteristics and by microscopic examination (400×). The bacterium was recovered daily throughout the five day holding period. Fewer colonies (based on qualitative observations) of *B. thuringiensis* were isolated after five days than after the first two days. The bacterium was also recovered from the gut contents of the surviving adults after the five-day holding period.

The overwintering butterflies, under laboratory conditions, showed low sensitivity to the *B. thuringiensis* used, both at the recommended and at twice the recommended concentrations for control of lepidopterous larvae. The results suggest that the application of this biotic agent within or near the butterfly's winter habitat presents a minimal threat to their survival. Because a small percentage of the butterflies did succumb to this bacterium or its product (endotoxin), however, the use of *B. thuringiensis* near the butterfly's overwintering site should be minimized.

Assuming that *E. hyalinaria,* the defoliator of fir trees, is equally susceptible to *B. thuringiensis* and transgenic *P. fluorescens,* the latter may be a better choice for controlling this insect. Our data suggest that the butterflies were less susceptible to the endoxotin alone than to a bacterial spore endotoxin mixture. To ensure another level of safety for the roosting butterflies from possible drift of the bioinsecticide, a spray-free buffer zone could be established around the overwintering site. This buffer zone, however, may be subjected to defoliation by *E. hyalinaria,* and could affect the roosting site. The possible threat of introducing the bacterium or its endotoxin into the protected aggregation sites by affected adults (via fecal droppings) exposed to the treated areas is minor due to the dilution effect, the poor survivability of the spores on the leaf surfaces (Leong, K. et al. 1980. Environ. Entomol., 9: 593–599) and the low adult susceptibility to *B. thuringiensis.* Another possible threat is the contamination of the monarch butterfly eggs with *B. thuringiensis* deposited by affected adults (Ali, A. A. & T. F. Watson. 1982. J. Econ. Entomol., 75: 596–598). Presumably, the overwintering butterflies will have eliminated most of the bacterium before egg deposition occurs. The monarch larvae sensitivity to *B. thuringiensis* from adults remains unknown and requires further study.

Although our study suggests that *B. thuringiensis* presents a minimal threat to the adult monarch butterflies, the susceptibility of the populations while in Mexico

still needs to be investigated before wide scale spraying is employed near their winter aggregation site.

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# NEW DISTRIBUTIONAL RECORDS FOR SOME CANDIDATE SPECIES OF *LYTTA* IN CALIFORNIA (COLEOPTERA: MELOIDAE)

Five Californian species of *Lytta* blister beetles (Coleoptera: Meloidae) are candidates for listing as endangered (i.e., a species that is likely to become extinct) by the U.S. Fish and Wildlife Service. These species [*L. hoppingi* Wellman, *L. insperata* (Horn), *L. moesta* (Horn), *L. molesta* (Horn), and *L. morrisoni* (Horn)] have not been listed due to a lack of biological information. Developments in California's central valley and surrounding foothills continue to impact them and their habitat because of three factors: our knowledge of their biology is limited, a lack of adequate survey methods, and the beetles not being fully protected by the Endangered Species Act (1973).

Recently, specimens of four of these candidate species were found in the R. S. Wagner Collection at the Tulare County Agricultural Commissioner's/Sealer's Office, Visalia, California. The specimens represent new distributional records for three species, having been collected in the 1930s but overlooked by researchers. For two of these species, an additional, more recent, distributional record is reported. We present this information (see Records section) to improve and update the distributional data on *Lytta*; to aid federal, state, and county regulatory agencies in their development, management, and protection efforts; and to encourage researchers to utilize the Wagner Collecton and to study the endangered invertebrate fauna. Distributional information on *Lytta* has been summarized (Selander, R. B. 1960. Illinois Biol. Monographs, 28) and recently updated (California Department of Fish and Game. 1991. Natural Diversity Data Base, computer data base of sensitive species. Sacramento, California).

Biological information for the five candidate species of *Lytta* is almost nonexistent. *Lytta molesta* has been collected on *Lupinus* (Leguminosae) feeding on